COMPARISON OF THE EFFECTS OF PROPRANOLOL AND ICI 66082 IN BLOCKING THE RENIN RELEASING EFFECT OF RENAL NERVE STIMULATION IN THE CAT

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The comparative effectiveness of propranolol and ICI 66082, in inhibiting the release of renin from the kidney resulting from renal nerve stimulation, has been studied in the cat. Over the dose range 0.17-0.68 $\times 10^{-5}$ mol/kg propranolol there was a dose-response relationship with the increase of plasma renin activity (PRA) achieved after 10 min stimulation. Over the dose range 0.75-1.69 $\times 10^{-5}$ mol/kg ICI 66082 also inhibited the release of renin caused by nerve stimulation, but was about five times less effective than propranolol. It would appear from these data that the β-adrenoceptors within the kidney mediating renin release are distinctly different from those of the heart.

It is becoming increasingly clear that the action of propranolol in inhibiting renin release from the kidney is due to its β-adrenoceptor blocking activity. Many studies have shown that propranolol can reduce renin production in situations of catecholamine release (Assaykeen, Clayton, Goldfein & Ganong, 1970), catecholamine administration (Ueda, Yasuda, Takabatake, Iizuka, Iizuka, Ihori & Sakamoto, 1970; Meurer, 1971) and by renal nerve stimulation (Vander, 1965; Coote, Johns, Macleod & Singer, 1972; Loeffler, Stockigt & Ganong, 1972). Further, (+)-propranolol, the isomer having no β-receptor blocking activity, is unable to block the rise in renin resulting from either infusion of isoprenaline (Assaykeen & Tanigawa, 1972; Van Dongen, Peart & Boyd, 1973) or from neurally stimulated release (Tobert, Slater, Fogelman, Lightman, Kurtz & Payne, 1973; Johns & Singer, 1974).

The characteristics of the β-adrenoceptor leading to renin release have not been fully investigated, although two recent reports, employing cardioselective β-receptor blocking drugs, practolol (Esler & Nestel, 1973) and ICI 66082 (Aberg, 1974) have shown that they are capable of reducing renin release from the kidney. An approach to this problem has been made in the present study in which we have stimulated the renal nerves of the cat and compared the renin-inhibiting activity of propranolol, a non-selective β-adrenoceptor blocking drug, with that of ICI 66082, a cardioselective blocking drug, at several dose levels.

Methods Male cats, weighing from 2.9 to 4.5 kg, were anaesthetized with sodium pentobarbitone 42 mg/kg intraperitoneally initially, with further small doses administered intravenously as necessary. Measurement of blood pressure, heart rate, route of drug administration and measurement of renal blood flow were as described previously (Johns & Singer, 1974). In all experiments the right kidney was removed by a retroperitoneal approach and the left kidney was similarly exposed.

To overcome any fall in blood pressure resulting from administration of the blocking drugs, a cotton thread was passed around the aorta approximately 2 cm below the left renal artery, and tightened as necessary.

Dissection of the renal nerves was as described by Coote *et al.* (1972). A 10 min period of stimulation was used with square wave stimuli of 15 V, 15 Hz and 0.2 ms duration obtained from a stimulator (Grass S8).

The first blood sample was taken at least 2 h after the completion of denervation and not less than 30 min after the start of the continuous infusion of the blocking drug. Blood samples were assayed for renin as previously described (Coote *et al.*, 1972) except that 2 ml blood samples were taken instead of 3.5 ml in most instances. The volume of the samples was replaced by the cells of the previous sample appropriately diluted with dextran. Results are expressed as ng angiotensin I generated per ml plasma per hour. Changes in the level of PRA are interpreted as a reflection of changes in the rate of renin secretion from the remaining kidney.

Both blocking drugs were dissolved in 0.9% w/v NaCl solution (saline). They were injected intravenously over a period of 15-20 min in a volume of 1 ml/kg. Then a continuous infusion of the drug was administered over the course of the experiments (initial dose/h in 6 ml). The drugs used were (±)-propranolol hydrochloride (ICI Macclesfield) and ICI 66082 (4-(2′-hydroxy-3′-iso-

propylamino-proproxy)phenyl acetamide, ICI Macclesfield).

Up to four experiments were carried out in any one animal. Each experiment consisted of three blood samples. The first, taken 10 min before the start of stimulation, the second 10 min after the start of stimulation and the third 20 min later. An interval of 30 min was allowed between experiments.

Results The effect of administration of propranolol and ICI 66082 on the PRA levels before, after 10 min renal nerve stimulation and 20 min after the end of stimulation is shown in Figure 1a. At least three animals were used at each dose level. Propranolol was found to reduce the initial levels of PRA in a dose-dependent manner and, although ICI 66082 also decreased the initial PRA values, the decrease was not related to the dose of drug administered. Both propranolol and ICI 66082 decreased the values of PRA achieved after 10 min of renal nerve stimulation, a 50% reduction in the response was obtained with the lowest dose of propranolol used, 0.17 x 10^{-5} mol/kg.

In order to quantify the degree of inhibition by these blocking drugs, the increase in PRA values was calculated which would take into account the different baselines of PRA at the different doses of drug. This value was obtained by subtracting the pre-stimulation PRA value from that obtained after 10 min of stimulation at the particular dose level of drug. These increases in PRA were used to construct a log dose-response curve and the results are given in Figure 1b. For propranolol the line joining the points is the calculated line of best fit. However, for ICI 66082 a line has been drawn to join the mean values of increase in PRA. The log dose-response curves have been used to calculate a ratio between the two drugs which relates to their differing potency of blockade. Because the lines are not quite parallel this ratio has been calculated in a range where the response to renal nerve stimulation was high and where it was low. Thus, with an increase in PRA of 7.0 ng ml^{-1} h^{-1} resulting from renal nerve stimulation, the propranolol to ICI 66082 ratio was 1 : 6.40; with an increase of 4.5 ng ml^{-1} h^{-1}. a ratio of 1 : 4.69 was obtained. From these two values the mean ratio was calculated as 1 : 5.55. Thus ICI 66082 was approximately 5.55 times less effective than propranolol in blocking renin release resulting from renal nerve stimulation.

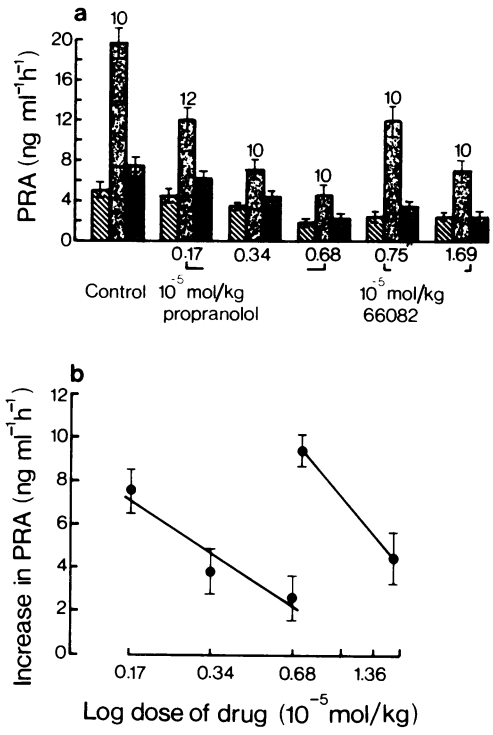

Fig. 1 A comparison of the plasma renin activity (PRA) response to 10 min of renal nerve stimulation with different doses of propranolol and ICI 66082 (a) showing values before (diagonally hatched areas), during (stippled areas), and after (cross hatched areas) stimulation; and (b) showing the log dose-response curves for the two drugs. Number of experiments is given above the column.

Discussion We have chosen electrical stimulation of the cut ends of the renal nerves as a standard way of stimulating renin release in order to compare the inhibitory action of propranolol and ICI 66082. This method has the advantage of being localized to the tissue under study and therefore has no peripheral effects, such as might be evident with infusion of catecholamines (Reid, Schrier & Earley, 1972), yet it causes the release of renin through the mediation of noradrenaline within the kidney. The response to renal nerve stimulation showed great variation from animal to animal, measured not only by renal blood flow changes, but also measured by the degree of rise in renin. However, when the renin log dose-response curves for the two drugs are compared, they are parallel within the limits of experimental error, indicating that the drugs are probably acting on the same receptor.

Propranolol, even at the lowest dose administered, 0.17 x 10^{-5} mol/kg, was found to block almost 50% of renin release in response to renal nerve stimulation. This dose of drug was in

the range used by Leoffler *et al.* (1972) ($0.17\text{-}0.34 \times 10^{-5}$ mol/kg) and Passo, Assaykeen, Goldfein & Ganong (1971) ($0.14\text{-}0.20 \times 10^{-5}$ mol/kg) in dogs, but is much less than we used in previous studies (Coote *et al.*, 1972; Johns & Singer, 1973). However, in these earlier studies we gave sufficient propranolol to block completely not only the release of renin, but also the increase in heart rate caused by at least 1 μg/kg of isoprenaline which is 10 times the amount normally required to produce a clear cut rise in heart rate. It is interesting to see from our present results (Fig. 1b) that for complete blockade of renin release resulting from renal nerve stimulation, a dose of at least 1.25×10^{-5} mol/kg is required which is very close to the value we originally used.

ICI 66082 is a newly developed cardioselective β-adrenoceptor blocking drug of approximately equal potency with propranolol on the heart, but between 14 and 40 times less potent on other peripheral β-receptors and is without sympathomimetic or membrane stabilizing properties (Barrett, Carter, Fitzgerald, Hull & Le Count, 1973). It is therefore a useful tool in studying the specificity of the β-receptor mediating renin release. There is little information available on this point at present. Recently Esler & Nestel (1973) administered practolol, a less potent cardioselective drug, to hypertensive patients, and observed a reduction in both normal renin levels and the levels reached in response to tilting. However, they made no attempt to compare this effect with that of propranolol. Similarly, Aberg (1974), who administered ICI 66082 to hypertensive patients, showed it to reduce renin, but did not compare its effectiveness with that of propranolol. In the present study we have evaluated the potency of propranolol over a range of doses and have shown that the cardioselective β-receptor blocker is about five times less effective than propranolol. This suggests strongly that the β-receptor concerned with the release of renin is distinctly different from the β-receptors in the heart. Other studies in this laboratory, with isolated renal cortical cells, and a variety of sympathomimetic amines, have shown that the renin releasing β-adrenoceptor may be more like that of the peripheral vascular beds (Johns, Richards & Singer, 1974).

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